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Molecular characterization of Tunisian B-acute lymphoblastic leukemia

Abstract

B-Acute lymphoblastic leukemia (B-ALL) represents a heterogeneous spectrum of lymphoid disorders and stands as the most common hematological malignancy affecting both children and adults. The diagnosis generally based on morphological criteria as well immunophenotyping, while molecular approaches provide highly valuable clinical and prognostic information. In this study, our aim was to investigate *IGH*, *IGK-Kde*, and *IKZF1* genes as molecular markers to enhance the accuracy of B-ALL diagnosis. Therefore we explored 63 B-ALL Tunisian cases, using multiplex PCR assay according to BIOMED-2 condition. 34 clonal *IGH* gene rearrangements, 22 clonal *IGK-Kde*, 4 *IKZF1* gene deletions and 2 simultaneous *IG/IKZF1* recombination were identified. These findings confirm both the clonal proliferation and the B-lymphoid lineage origin. The use of *IGH*, *IGK-Kde* and *IKZF1* markers will be introduced for the first time in Tunisian laboratories for molecular characterization of B-ALL and subsequently for the monitoring of minimal residual disease which is an important determinant for patients outcome. The implementation of molecular profiling of B-ALL trough assessing *IGH*, *IGK-Kde*, and *IKZF1* markers will rise the challenge for efficient minimal residual disease monitoring and patient outcomes evaluation.

Keywords: B-ALL, IGH rearrangement, IGK rearrangement, IKZF1 deletion, multiplex PCR

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Introduction

Acute lymphoblastic leukemia (ALL) is a malignant disorder originating from lymphoid progenitor cells committed to differentiating into T or B cells. Its origin is attributed to significant genetic abnormalities in precursor blood cells. ALL is the most common hematological malignancy with B-cell origin (B-ALL) with predominance ranging from 75% to 92%.[1] Assessing clonality through targeted molecular markers can significantly support the diagnosis of B-ALL. Studies highlight that 5 to 15% of leukemia cases could benefit from molecular clonality assessment.[2] Unlike cytomorphology, molecular biology approach aligned with immunophenotyping, allow best characterization of leukemic blasts by confirming the monoclonality and the B-lineage origin of the malignant proliferation. Several molecular methods have been developed to identify leukemic population. The immunoglobulin (IG) gene rearrangements are the most widely applied targets. Heavy chain (*IGH*) and light chain (*IGK*/*IGL*) genes recombination are commonly used for B-leukemia characterization.[3,4] During the *IGH* locus rearrangement, the $V_HD_HJ_H$ recombination involves one of the V_H gene from the seven subgroups (*IGHV1-IGHV7*). The similarity in rearrangements among these subgroup members primarily arises from their high sequence homology. This similarity enables the amplification of the *IGH* gene using consensus primers via molecular methods.[5–7]

For *IGL* locus rearrangement, the *IGK* locus is deleted involving the kappa deleting element (*Kde*). Two different types of *Kde* recombination can inactivate the *IGK* rearrangements: *Kde* rearranges to the intron *RSS* leading to the deletion of the *IGKC* gene and the maintain of the *IGKV-J* junction, or *Kde* rearranges to one of the *VK* genes leading to the exclusion of constant region and intron RSS with the subsequent loss of the *IGKV-J* junction.[8,9] Therefore, it is possible to detect both *IGKV-J* and intron-*Kde* deletional rearrangements on the same allele.[8,9]

During each *IGH* or *IGK* recombination event, nucleotides are inserted at the junctions of *VDJ* or *VK-Kde/RSS-Kde*, respectively. This process creates N-regions, which serve as unique molecular sequence tags specific to each lymphocyte. These tags are crucial for identifying and characterizing the diversity of lymphocytes based on their specific genetic rearrangements.[10,11] Since almost 90% of B-ALL rearrange the *IGH* locus and 50% delete the *IGK-Kde* locus, these molecular markers are commonly used for of B-leukemia diagnosis.

Similarly, the Ikaros protein mediates chromatin accessibility necessary for *VDJ* recombination add to modulates the expression of early B-cell-specific genes.[12] Ikaros is encoded by Ikaros Family Zinc Finger 1 gene (*IKZF1*) and contains variable number of Kruppel-like zinc fingers in two domains involved in DNA binding.[13] *IKZF1* deletions are closely related to the development of lymphoid leukemia and found in nearly 30% B-ALL Ph^-.[11] The most common deletions (~45%) occur in exons 4 to 7. The 5' and 3' breakpoints are highly conserved and are flanked by recombination signal sequences RSS. This suggests that this deletion is mediated by off-target effects of the recombination activating gene during *VDJ* recombination in B cell progenitors.[14,15] The deletions Δ4-7 and Δ2-7 are the most frequently identified. *IKZF1* deletions are associated with a poor prognosis in pediatric and adult B-ALL and can be an efficient predictor of relapse.[16]

Patient risk stratification may be improved by comparing *IKZF1* status to other adverse prognosis criteria.[16] The B-ALL diagnosis is based on a combination of clinical assessment, cytological examination, cytogenetic analysis, and immunophenotypic classification. These approaches may often present some difficulties of interpretation,

therefore, more sensitive and complementary methods are requested for leukemia diagnosis and patients' treatment stratification. We suggest utilizing a combination of molecular markers, such as *IKZF1* deletions and *IGH/IGK-Kde* rearrangements, to enhance the diagnosis of B-ALL in Tunisian patients."

Material and methods

Patients

63 Tunisian B-ALL cases were included in this study. 44 B-ALL were diagnosed at Hematology department, F. Hached Hospital of Sousse and 19 diagnosed at Biological Hematology department, A. Othmana Hospital of Tunis. Informed consent was obtained from all patients or their legal guardian as required by the Helsinki Declaration. The cohort of patients consisted of 38 males and 25 females with sex ratio: 1,52 and median age of 8 years at the time of diagnosis (1-57 years). The B-ALL were characterized based on the French-American-British classification and flow cytometric immunophenotyping.

Mono nuclear cells and DNA isolation

Samples were collected from either bone marrow (23 patients) or peripheral blood (40 patients) upon diagnosis, where blast count exceeded 75%, ensuring detectable analysis of *IGH* and *IGK-Kde* rearrangements, as well as *IKZF1* deletions. Mononuclear cells were isolated using Ficoll-Paque density centrifugation and were subsequently utilized for DNA extraction. Genomic DNA extraction was performed using either phenol chloroform or the QIAGEN DNA Blood kit as per the manufacturer's protocols. DNA quantity was determined using spectrophotometry, and its quality was assessed via agarose gel electrophoresis.

PCR analysis

Clonal *IGH* and *IGK-Kde* gene rearrangements and *IKZF1* deletion were investigated by multiplex PCR amplification. For *IGH* gene rearrangements, *VH* family specific primers combined with a consensus *JH* primer were used.[17] For *IGK-Kde* rearrangements, a reverse Kde primer was used in combination with one of six *VK* family-specific primers or with *RSS* primer located at the *JK-CK* intron.[17] For *IKZF1* deletion, specific Δ2-7, Δ2-8, Δ4-7 and Δ4-8 primers we used as previously described (primers are available under request).[16] PCR amplifications were conducted in triplicate following the BIOMED-2 consortium protocol.[16] PCR products were subsequently identified using Gene Scanning on the ABI Prism 3130xl Genetic Analyzer (Applied Biosystems).

Results

Clinical and biological characteristics

The hyperleukocytosis observed in 63 patients in favor of B-ALL clinical suspicion that reflects the high tumor burden. Cytomorphological examination highlighted transient massive blastosis in peripheral blood, along with bone marrow infiltration by leukemic cells and extramedullary manifestations. The myelogram revealed more than 20% blasts, with immunophenotyping indicating low CD45 intensity, allowing gating of blasts expressing HLA-DR and CD34. B-ALL cases were positive for CD79a, CD22, and CD19, classified predominantly as B-ALL III according to EGIL classification.

Molecular characteristics

The validity of the extracted leukemic DNA was confirmed by RQ-PCR, as the obtained threshold cycle (Ct) differed by no more than ±1 Ct from the control DNA (with Ct values of 23.9 for 50ng and 20.5 for 500ng of DNA). Only 55 genomic DNAs among 63 were investigated for IGH / IGK-Kde and IKZF1 loci analysis due inadequate DNA quality. The multiplex PCR was performed using standard primers specific to either *IGH* or *IGK-Kde* loci. 56 clonal *IGH* and *IGK-Kde* rearrangements were revealed. For *IGH* rearrangement assessment, using the combination of 7 primers (*VH1* to *VH7*) via multiplex PCR assay, 34 B-ALL *VH-JH* rearrangement were revealed with one pic at the expected size 250-295 pb (Figure 1). The majority of rearrangements included *VH3*, *VH4* genes; followed by *VH1*, *VH2*, *VH5* and *VH7*. For *IGK-Kde* rearrangement, a combination of 7 primers (*VK1f/6* to *VK7* and intron *RSS*) via multiplex PCR assay; In 22 B-ALL, we obtained one pic at the expected size of 120-300 pb corresponding to *VK-Kde* or *RSS-Kde* rearrangement (Figure 2). The *VK1f/6-Kde* rearrangement was predominant one. The recombination events involving *Kde* were found to include *VK2*, *VK3*, *VK7*, and the intron RSS sequences. Four B-ALL cases showing bi-allelic *IGK-Kde* rearrangements: two cases revealed *VK1f/6-Kde* on the first allele and *VK7-Kde* on the second one, and two cases exhibited *VK3-Kde* on one chromosome and intron *RSS-Kde* on the second allele. Concomitant rearrangements of both *IGH* and *IGK* loci were observed in 14 B-ALL cases. The investigation of *IKZF1* gene was performed in 39 cases by targeting Δ2-7, Δ2-8, Δ4-7 and Δ4-8 deletions using multiplex PCR assay. We obtained one pic at the expected size of 120-195 pb corresponding to the Δ4-7 deletion identified in 3 B-ALL and one pic at the expected size of 250-280 pb corresponding to the Δ2-8 deletion (Figure 3).

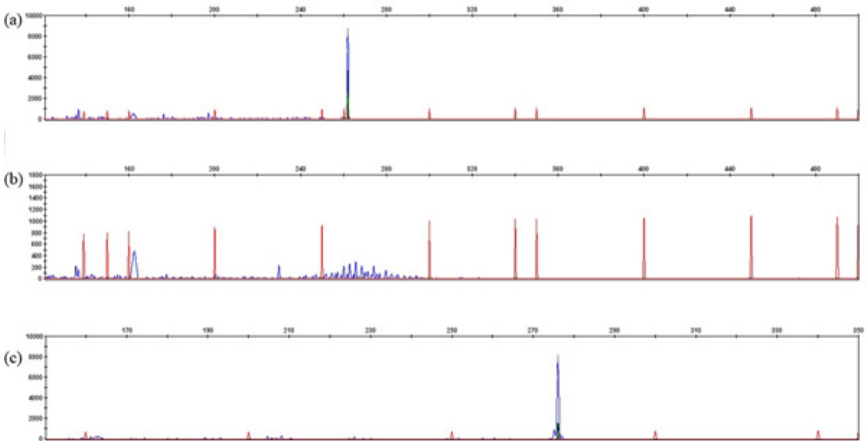

Figure 1 Gene scan of IGH rearrangements: a) monoclonal control DNA, b) polyclonal control DNA, c) monoclonal IGVH$_1$(P14).

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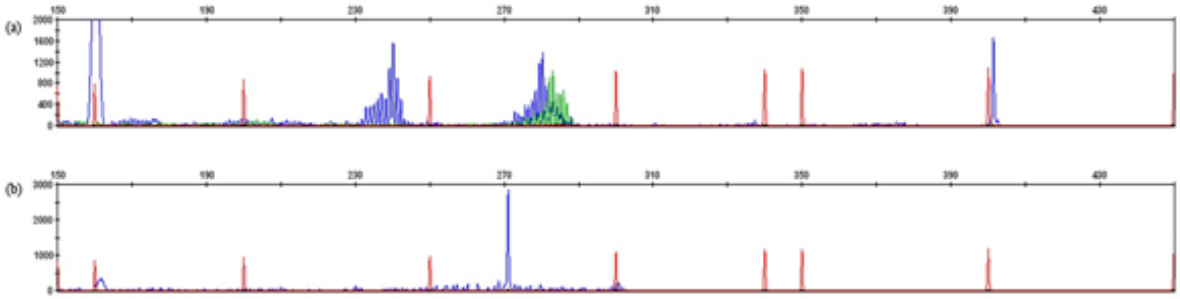

Figure 2 Gene scan of IGK-Kde rearrangements: a) polyclonal VK3-Kde and Intron RSS-Kde, b) control VK3-Kde.

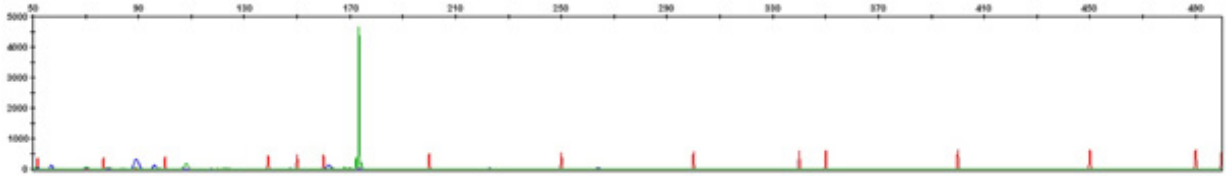

Figure 3 IKZF$_1$ deletion: Δ4-7.

Discussion

B-ALL represents the predominant subgroup among hematological malignancies, affecting both children and adults (comprising 60% to 80% of all cases of ALL). For a comprehensive characterization of leukemic lymphoblasts, it is essential to integrate cytological diagnosis and immunophenotyping with molecular approaches. This ensures the most accurate classification and understanding of the disease. Through our study, we introduced the molecular diagnosis approaches for accurate diagnosis and monitoring of B lineage leukemias, since this method is not commonly used in laboratories. Among standard molecular markers, PCR-based analysis of immunoglobulin rearrangements has gradually become a gold standard method. *IGH*, *IGK-Kde* rearrangements can be commonly used for B-ALL diagnosis since they are identified in nearly 90% and 50% of cases respectively.[18,19] Moreover, *IKZF1* is deleted in 28% of B-ALL and shows poor prognostic mainly in B-ALL Ph$^-$.[20] Therefore, we combined *IGH* / *IGK-Kde* rearrangements and *IKZF1* deletions as molecular markers for the first time to assess Tunisian B lineage leukemia.

We have analyzed 63 B-ALL cases based on cytomorphological criteria and immunophenotyping. The *IGH* and *IGK-Kde* multiplex PCR assays were performed using BIOMED-2 conditions. The presence of clonal proliferation was confirmed in 34 B-ALL through *IGH* rearrangements. For $V_{H}J_{H}$ rearrangement, the *VH* gene involvement was similar to previous reported studies. The VH3 and VH4 were the most commonly genes involved (26% vs 20%-50%), followed by VH1 (17% vs 10%-20%), VH2 (6% vs 5%-11%) and VH5 (3% vs 3%-4%).[21] The VH6 gene rearrangement was not detected in our investigated cases despite it is frequently rearranged in B-ALL according literature.[17] We have demonstrated the *IGK* gene deletions in 22 cases of B-ALL, characterized by *Kde* segment rearrangements predominantly involving the *Vκ1f/6* gene (50% vs 68%-100%), and less frequently with intron RSS (14% vs 0-31%).[3,17,22]

In our cohort, 64% of B-ALL involved concomitant *IGH* and *IGK-Kde* rearrangements. These two combined genetic markers are therefore two powerful and helpful marker for the clonality assessment and the accurate diagnosis for the vast major of B-ALL subtypes. Using multiplex PCR, the deletion of *IKZF1* gene were detected in 10% of B-ALL investigated cases compared to 9%-20% as described in literature.[23] Therefore, we have verified that the lymphoblastic proliferation is clonal, with the Δ4-7 deletion in *IKZF1* identified as the most frequent deletion. Most *IKZF1* intragenic deletions including exons 4 to 7, drive the expression of a non-DNA binding Ikaros isoform with dominant negative activity. This implies an active, ongoing recombination at *IKZF1* deletion junction during the leukemogenic process.[23,24] The significant correlation between *IKZF1* mutations and poor prognosis has led to recommendations for screening *IKZF1* mutations upon diagnosis.[16] In children, *IKZF1* deletions are a better predictor of relapse compared to conventional classification based on clinical and cytogenetic criteria.[24] This suggests that risk stratification of patients could be enhanced by comparing *IKZF1* status with other adverse prognosis criteria, such as residual disease status. Interestingly, in our study, two B-ALL cases exhibited simultaneous modifications in the *IGH*, *IGK-Kde*, and *IKZF1* genes. These findings strongly support the diagnosis of clonality and suggest potential for improved patient stratification to enhance follow-up and refine therapeutic protocols.

Conclusion

Here, we have employed a molecular approach to successfully detect combined IGH/IGK-Kde rearrangements and *IKZF1* deletions for diagnosing B-ALL patients. We aim imply this approach in Tunisian laboratories. The tags of both *IG* and *IKZF1* would be a helpful markers for best characterization of B-ALL to complete cytomorphology and immunophenotyping approaches. The loss of *IKZF1* may interact with *IGH* rearrangements and other biological or clinical characteristics to define subsets of leukemia that are particularly aggressive. When *IKZF1* mutations are detected at diagnosis, they may be reliable markers to follow minimal residual disease (MRD).[16,23] We will further introduce both *IG* and *IKZF1* genes to follow-up B-ALL patients during treatments and assess the MRD which is powerful prognosis indicator.

Ethical standards

This study was approved by the ethics committee of the hospital and all subjects gave their informed consent according to Helsinki declaration.

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Conflicts of interest

The author declares that there are no conflicts of interest.

References

1. Seth R, Singh A. Leukemias in children. *Indian J Pediatr*. 2015;82(9):817–824.
2. Langerak AW, Groenen PJTA, Bruggemann M, et al. Euro clonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. *Leukemia*. 2012;26(10):2159–2171.
3. Beishuizen A, Verhoeven MA, Mol EJ, et al. Detection of immunoglobuline kappa light-chain gene rearrangement patterns by southern blot analysis. *Leukemia*. 1994;8(12):2228–2236.
4. Giudicelli V, Chaume D, Lefranc MP. IMGT/V-QUEST, an integrated software program for immunoglobulin and T cell receptor V-J and V-D-J rearrangement analysis. *Nucleic Acids Res*. 2004;32:W435–W440.
5. Deane M, Norton JD. Immunoglobulin gene 'fingerprinting': an approach to analysis of B lymphoid clonality in lympho proliferative disorders. *Br J Haematol*. 1991;77(3):274–281.
6. Kuppers R, Zhao M, Rajewsky K, et al. Detection of clonal B cell populations in paraffin-embedded tissues by polymerase chain reaction. *Am J Pathol*. 1993;143(1):230–239.
7. Cook GP, Tomlinson IM. The human immunoglobulin VH repertoire. *Immunol Today*. 1995;16(5):237–242.
8. Brauninger A, Goossens T, Rajewsky K, et al. Regulation of immunoglobulin light chain gene rearrangements during early B cell development in the human. *Eur J Immunol*. 2001;31(12):3631–3637.
9. Langerak AW, Nadel B, De Torbal A, et al. Unraveling the consecutive recombination events in the human IGK locus. *J Immunol*. 2004;173(6):3878–3888.
10. Dongen JJM, Wolvers T. Analysis of immunogobulin and T-cell receptor genes: Basic and technical aspects. *Clin Chim Acta*. 1991;198(1–2):1–92.
11. Gill JI, Gulley ML. Immunoglobulin and T-cell receptor rearrangement. *Hematol Oncol Clin North Am*. 1994;8(4):751–770.
12. Thompson EC, Cobb BS, Sabbattini P, et al. Ikaros DNA-binding proteins as integral components of B cell developmental-stage-specific regulatory circuits. *Immunity*. 2007;26(3):335–344.
13. Yoshida T, Ng SY, Zuniga JC. Early hematopoietic lineage restrictions directed by Ikaros. *Nat Immunol*. 2006;7(4):382–391.
14. Kastner P, Dupuis A, Gaub MP, et al. Function of Ikaros as a tumor suppressor in B cell acute lymphoblastic leukemia. *Am J Blood Res*. 2013;3(1):1–13.
15. Mullighan CG, Miller CB, Radtke I, et al. BCR-ABL$_1$ lymphoblastic leukaemia is characterized by the deletion of Ikaros. *Nature*. 2008;453(7191):110–114.
16. Caye A, Beldjord K, Mass MK, et al. Breakpoint-specific multiplex PCR allows the detection of IKZF1 intragenic deletions and minimal residual disease monitoring in B-cell precursor acute lymphoblastic leukemia. *Haematologica*. 2013;98(4):597–601.
17. Dongen JJM, Langerak AW, Bruggemann M, et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH$_4$-CT$_{98}$-3936. *Leukemia*. 2003;17(12):2257–2317.
18. Beishuizen A, Verhoeven MA, Mol EJ, et al. Detection of immunoglobulin Kappa light-chain gene rearrangement patterns by Southern blot analysis. *Leukemia*. 1994;8(12):2228–2236.
19. Stolz F, Panzer S, Panzer ER. Multiplex PCR reaction for the detection and identification of immunoglobulin kappa deleting element rearrangements in B-lineage leukemias. *Br J Hematol*. 1999;106(2):486–494.
20. Cazzaniga G, Van FW, Lo NL, et al. Developmental origins and impact of BCR-ABL$_1$ fusion and IKZF1 deletions in monozygotic twins with Ph+ acute lymphoblastic leukemia. *Blood*. 2011;118(20):5559–5564.
21. Mortuza FY, Moreira IM, Papaioannou M, et al. Immunoglobulin heavy-chain gene rearrangement in adult acute lymphoblastic leukemia reveals preferential usage of J(H)-proximal variable gene segments. *Blood*. 2001;97(9):2716–2726.
22. Szczepanski T, Langerak AW, Wolvers ILM, et al. Immunoglobulin and T cell receptor gene rearrangement patterns in acute lymphoblastic leukemia are less mature in adults than in children: implications for selection of PCR targets for detection of minimal residual disease. *Leukemia*. 1998;12(7):1081–1088.
23. Mullighan CG, Su X, Zhang J, et al. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. *N Engl J Med*. 2009;360(5):470–480.
24. Waanders E, van VH, Schoot CE, et al. Integrated use of minimal residual disease classification and IKZF$_1$ alteration status accurately predicts 79% of relapses in pediatric acute lymphoblastic leukemia. *Leukemia*. 2011;25(2):254–258.